Dynamics of Oestrogen-Receptor Distribution Between the Cytosol and Nuclear Fractions of Immature Rat Uterus after Oestradiol Administration

By J. MEŠTER and E. E. BAULIEU

*Unité de Recherches sur le Métabolisme Moléculaire et la PhysioPathologie des Stéroïdes de l'Institut National de la Santé et de la Recherche Médicale, Université Paris-Sud, Département de Chimie Biologique, Hôpital de Bicêtre, 78 rue du Gl Leclerc, 94270-Bicêtre, France**

(Received 18 October 1974)

1. The nuclear-myofibrillar (800g pellet) fraction of the uterus from immature (22-23 days old) rats not exposed to oestrogen exhibits saturable binding of oestradiol. This nuclear binding capacity represents approximately 10% of that of the cytosol fraction (approx. 3.5 fmol/ μ g of DNA). The predominant part (0.3 fmol/ μ g of DNA) of the nuclear binding sites are present in the residual pellet after extraction with 0.5M-KCl. 2. By using an exchange technique *in vitro*, determinations of the nuclear binding sites have been carried out after administration of 1 μ g of oestradiol *in vivo*. Within 0.5h after the hormone injection, the concentration of nuclear binding sites increased to approx. 0.4 fmol/ μ g of DNA in the 0.5M-KCl-extractable fraction, and to approx. 1.2 fmol/ μ g of DNA in the residual fraction. Meanwhile the cytosol oestrogen-receptor concentration decreased to approx. 10% of its initial value. In the following period from 0.5h after the oestradiol injection onwards, the concentration of nuclear oestrogen receptors decreased with half-life values of approx. 140 and 200min for the KCl (0.5M)-extractable and residual form respectively. At the same time, the cytosol receptor concentration increased to reach approx. 50% of the initial value by 6h. This increase could not be blocked by cycloheximide. The initial concentration of cytosol receptor was restored approx. 11h after the injection and the increase during the 6-11h period was sensitive to cycloheximide inhibition, suggesting protein-synthesis-dependence of the process. 3. With the (more) physiological dose of oestradiol (0.1 μ g), the decrease of the cytosol receptor was only 50% by 4h and this was followed by a period (up to 12h after injection) during which the initial concentration was restored. During this period the increase of the receptor can be blocked by cycloheximide.

Injection of oestradiol into immature rats rapidly leads to a decrease of the concentration of uterine cytosol high-affinity oestrogen-binding sites (receptor) and to an increase of the nuclear oestrogen receptor concentration (e.g. per total uterus). With the so-called physiological dose of the hormone (0.1 μ g/animal), the decrease of the receptor content 4h after injection is approx. 50% of the initial value (Sarff & Gorski, 1971), and this loss is only partly accounted for by the increase of the nuclear receptor concentration (Feherty, 1972; Milgrom *et al.*, 1973). The cytosol receptor concentration is restored during the following period reaching approx. 1.5 times its initial value by 24h after the injection; this process is inhibited by cycloheximide (Jensen *et al.*, 1969; Sarff & Gorski, 1971). The mechanism by which the cytoplasmic oestrogen receptor concentration is restored was examined by Sarff & Gorski (1971) whose results suggested that virtually all the protein

synthesis necessary is completed within approx. 4h after administration of oestradiol (0.1 μ g).

A different situation is seen with a higher dose (1 μ g) of oestradiol; the cytosol receptor concentration falls rapidly, the minimum value being reached within 30min (De Sombre, 1970). The present work aimed to study in quantitative terms the receptor distribution between the cytosol and nuclear fractions of immature rat uterus after the administration of the higher (1 μ g) dose of oestradiol and to re-examine under these circumstances the mechanism of restoration of the cytosol receptor concentration.

Materials and Methods

Steroids

[2,4,6,7-³H]Oestradiol-17 β (100Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; unlabelled oestradiol-17 β was a product of Roussel UCLAF, Romainville, France. Both

* Postal address: Lab Hormones, F94270-Bicêtre, France.

chemicals were found to be chromatographically pure. Solutions of [^3H]oestradiol were prepared and stored as described (Mešter & Baulieu, 1972); solutions of oestradiol were prepared by diluting the stock solution (0.4 mg/ml in ethanol) with the appropriate aqueous medium.

Animals

Female Wistar rats (22–23 days old) were used; they were injected with 1 or 0.1 μg of oestradiol in 0.5 ml of saline subcutaneously or intraperitoneally and killed by cervical dislocation at the time-intervals indicated. The uteri were removed immediately, cleaned of connective tissue and homogenized in a sucrose (250 mM)– MgCl_2 (3 mM) medium by using a glass–glass Kontes homogenizer. The tissues and homogenates were kept at 2–4°C. Three uteri were usually homogenized in 1 ml of medium.

Cell fractionation

The homogenates were centrifuged at 800g for 10 min to yield the low-speed supernatant fraction and the nuclear–myofibrillar pellet; the pellets were washed twice with 4 ml of medium and then resuspended in 0.5 ml of the homogenizing medium.

Measurement of oestrogen-binding capacity

Cytosol receptors. (a) Method (I). The low-speed supernatant fractions (0.1 ml) were incubated with [^3H]oestradiol at saturating concentration (13 nM) in the presence or absence of oestradiol (5 μM) in a total of 0.3 ml (steroids added in 0.2 ml of the homogenizing medium) for 30 min at 30°C. At the end of this period, 0.5 ml of charcoal (0.25%)–dextran (0.0025%, mol.wt. 90000) suspension in Tris buffer [10 mM–Tris–HCl (pH 7.4)–1.5 mM–EDTA] was added to each tube and the tubes were incubated for 10 min at 30°C to eliminate the free [^3H]oestradiol as well as a predominant part of the [^3H]oestradiol bound by the low-affinity sites (Mešter *et al.*, 1970). The suspensions were then centrifuged and the radioactivity of a portion (0.5 ml) of the supernatant was determined (see below). The concentration of the high-affinity oestrogen-binding sites was calculated after subtraction of the 'background' radioactivity (obtained in the presence of excess of oestradiol) from the total [^3H]oestradiol binding (i.e. in the absence of oestradiol). The concentration of [^3H]oestradiol used in this method to saturate the receptor binding sites was sufficiently high to eliminate the possible influence of endogenous oestradiol on the assessment of free binding sites, since the concentration of oestradiol (measured by competition binding technique, see below) at its maximum, i.e. 0.5 h after injection of 1 μg of hormone/rat, remained less than 2 nmol/litre of supernatant fraction.

(b) Method (II). Alternatively, a method described previously (Mešter *et al.*, 1970) was used where the number of high-affinity oestrogen-binding sites was determined by a Scatchard (1949) plot analysis. Portions (0.1 ml) of the uterine supernatant preparation were incubated with [^3H]oestradiol at various concentrations (0.2, 0.5, 1.0 and 2.0 nM) for 30 min at 30°C in a total volume of 0.2 ml. Charcoal–dextran (0.5 ml) was then added, the tubes were incubated subsequently for 10 min at 30°C, centrifuged and the radioactivity of the supernatant was determined. The results were corrected for radioactivity not adsorbed by charcoal in the absence of the uterine supernatant and represented in terms of bound/unbound hormone against amount of bound hormone. The concentration of receptor binding sites participating in the equilibrium was indicated at the intersection of the resulting straight line with the abscissa. Feherty *et al.* (1970) showed that the technique yields identical results for both low-speed and high-speed cytosol uterine supernatant preparations.

Nuclear receptors. The binding capacity of nuclear preparations was determined essentially as described by Anderson *et al.* (1972). Portions (0.2 ml) of the resuspended nuclear fractions (usually containing 50–100 μg of DNA) were incubated in a total volume of 0.6 ml containing [^3H]oestradiol (13 nM) with or without oestradiol (5 μM) in the homogenizing medium for 30 min at 37°C. The mixtures were then cooled, centrifuged (800g for 5 min) and the pellets washed once with 4 ml of 0.25% Triton X-100 in the homogenizing medium, twice with 4 ml of the homogenizing medium and finally extracted by a single freezing and thawing in 0.5 ml of Tris buffer containing 0.5 M–KCl. The extracts were centrifuged (1500g for 5 min) and the radioactivity of the supernatants was determined; the residual pellets containing the hormone bound by the 'insoluble' oestrogen receptor were extracted with 1 ml of acetone, the extracts were dried and their radioactivity was measured. The number of the saturable oestrogen-binding sites was calculated in the same way as described above (method I) for the low-speed supernatant fractions.

The exchange technique *in vitro* for determination of the binding capacity of the nuclear preparation was confirmed in the cell-free reconstituted system (Fig. 1). Uterine nuclei were incubated with pre-formed supernatant receptor–(non-radioactive) oestradiol complexes and their oestrogen-binding capacity was measured by the exchange technique. A comparison was made with another portion of the nuclear preparation that had been incubated under the same conditions with supernatant receptor–[^3H]oestradiol complexes. The results confirmed the validity of the exchange procedure *in vitro*.

The exchange technique *in vitro* for determination of the binding capacity of the nuclear preparation was confirmed in the cell-free reconstituted system (Fig. 1). Uterine nuclei were incubated with pre-formed supernatant receptor–(non-radioactive) oestradiol complexes and their oestrogen-binding capacity was measured by the exchange technique. A comparison was made with another portion of the nuclear preparation that had been incubated under the same conditions with supernatant receptor–[^3H]oestradiol complexes. The results confirmed the validity of the exchange procedure *in vitro*.

Fig. 1. Exchangeability of the nuclear-bound oestradiol

Uteri were homogenized and fractionated as described in the Materials and Methods section. Portions (0.2 ml) of the cytoplasmic fraction were preincubated for 20 min at 30°C with [³H]oestradiol (mixture A) or oestradiol (mixture B) at a final concentration of 16 nM in each case, or with [³H]oestradiol (16 nM) plus oestradiol (3 µM) in total volumes of 0.5 ml. Portions (0.1 ml) of the resuspended nuclear fraction (corresponding to approx. 0.8 uterus) were added to the above mixtures and incubation was carried out for 30 min at 37°C. The nuclear fraction incubated in mixture B was subsequently washed, resuspended in a total volume of 0.6 ml containing the same final concentration of [³H]oestradiol as above (13 nM) and incubated for 30 min at 37°C. The amount of [³H]oestradiol in the nuclear fractions and the concentration of saturable binding detected in the pellets after incubation with [³H]oestradiol (i.e. direct uptake in mixture A, and the concentration of exchangeable nuclear oestrogen-binding sites in the nuclei after incubation in mixture B) were determined as described in the Materials and Methods section. □, 0.5 M-KCl-soluble receptor fraction; ■, 0.5 M-KCl-insoluble receptor fraction. The results shown represent the mean of two duplicate determinations.

Determination of endogenous oestradiol

The oestradiol concentration in the uterine low-speed supernatant fraction was measured by mixing portions (0.1 ml) of the sample or of standard oestradiol solution (0–200 pg) in buffer with [³H]-oestradiol (20 pg in 0.1 ml of buffer), heating at 70°C (3 min) to inactivate the receptor molecules present and then carrying out a competitive binding procedure by using rabbit uterine cytosol as described previously (Mešter *et al.*, 1971). When only the receptor-bound oestradiol was to be measured, the samples were treated first by the charcoal-dextran suspension for 10 min at 30°C to eliminate the free

hormone as well as hormone bound by the low-affinity binding sites. It has been demonstrated (Mešter *et al.*, 1970; Mešter & Robertson, 1971) that the dissociation of oestradiol–receptor complex is negligible (<5%) within this period, whereas ≥90% of the oestradiol bound at zero time to the low-affinity sites is adsorbed by charcoal during the incubation at 30°C for 10 min.

Protein-synthesizing activity

This was measured by injecting the rats intraperitoneally with 2 µCi of [¹⁴C]leucine (40 µCi/mmol) and measuring the ¹⁴C radioactivity in the trichloroacetic acid (5%, w/v) precipitates of the uterine cytosol fractions; the precipitates were washed with 5% trichloroacetic acid and dissolved in NCS tissue solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.).

Radioactivity determinations

These were carried out in a Packard model 3310 liquid-scintillation spectrometer by using 0–0.5 ml of the same sample plus 10 ml of Omnifluor (NEN Chemicals, Frankfurt, West Germany) solution (0.4%) in toluene (cf. Mešter *et al.*, 1970). The efficiency of counting was approx. 45% for ³H and approx. 80% for ¹⁴C. When necessary, corrections for quenching were made by using external or internal standards.

DNA contents

These were measured by the method of Burton (1956).

Results

Distribution of oestrogen receptors in the uterus of control animals

The concentration of oestrogen receptor in the uterine low-speed supernatant fraction of 22–23-day-old rats as determined by both methods (I) and (II) was approx. 3.5 fmol/µg of DNA (approx. 0.9 pmol/uterus), in good agreement with previous reports (e.g. Gorski *et al.*, 1970; Feherty *et al.*, 1970).

The receptor had an affinity for oestradiol characterized by a dissociation constant of approx. 0.2–0.4 nM at 30°C as calculated from the Scatchard (1949) plots obtained by method (II).

The nuclear fraction of the immature rat uterus showed saturable uptake of oestradiol under the conditions described above (the Materials and Methods section). The contents of oestrogen-binding sites were approx. 0.06 fmol/µg of DNA in the KCl (0.5 M)-extractable fraction and approx. 0.3 fmol/µg of DNA in the residual fraction.

Fig. 2. Uptake of uterine cytoplasmic receptor- $[^3\text{H}]$ -oestradiol complex by the nuclei; time-dependence at 37°C

Uteri (15) were homogenized and the nuclear and cytoplasmic fractions prepared as described in the Materials and Methods section. The cytoplasmic receptor- $[^3\text{H}]$ -oestradiol complex was prepared by preincubation of the cytosol fraction (2.5 ml) with 40 nM - $[^3\text{H}]$ -oestradiol (2 ml) for 20 min at 30°C . Portions (0.4 ml) of this solution were then incubated at 37°C for the periods of time indicated with the nuclear suspension (0.1 ml corresponding to approx. 0.8 uterus). The incubation was terminated by cooling and centrifugation, and the $[^3\text{H}]$ -oestradiol present in the nuclear fraction was determined as described in the Materials and Methods section. The results shown are corrected for variations in the DNA content in individual tubes and represent average values of two duplicate experimental determinations. Δ , $[^3\text{H}]$ -Oestradiol in the 'soluble' nuclear fraction (0.5 M-KCl extract); $\circ$, $[^3\text{H}]$ -oestradiol in the insoluble residue.

Under the cell-free reconstituted conditions the relative proportions of the 0.5 M-KCl-extractable and residual receptors were dependent on the length of incubation. The nuclear uptake of $[^3\text{H}]$ -oestradiol (presumably together with the receptor) in the presence of the uterine supernatant fraction was rapid into the soluble form, reaching a maximum value (approx. $0.4\text{ fmol}/\mu\text{g}$ of DNA) within 5 min with a subsequent decrease (approx. 60% of the 5 min concentration remaining at 60 min), whereas the concentration of the bound hormone in the insoluble nuclear fraction approached a maximum (approx. $1.5\text{ fmol}/\mu\text{g}$ of DNA) value only between 30 and 60 min (Fig. 2).

Effect of oestradiol administration on the receptor distribution

Both methods (I) and (II) showed approx. 90% decrease in the concentration of cytosol oestrogen-binding sites within 0.5 h after the hormone ($1\mu\text{g}$) administration (Fig. 3a). At the same time, the concentration of soluble nuclear receptor rose to approx. 0.4 fmol of oestrogen-binding sites/ μg of DNA (12% of the initial cytoplasmic receptor content) and that of the insoluble nuclear receptor to approx. 1.2 fmol of oestrogen-binding sites/ μg of DNA (35% of the initial receptor content). The net increase in the total nuclear receptor content accounted, however, only for approx. 50% of the loss of initial cytosol receptor content.

Since the method used to determine the cytosol receptor does not account for the occupied binding sites, the possibility that the missing receptor was present in the cytoplasm in the form of a complex with the hormone was examined. The concentration of total oestradiol in the low-speed supernatant fraction (related to the number of oestradiol molecules/cell) reached approx. 115% of the initial supernatant receptor content at 0.5 h after the hormone administration (assuming one binding site of the receptor equivalent to one oestradiol molecule). However, when the oestradiol determination was performed on the charcoal-treated (10 min at 30°C) low-speed supernatant fraction, no detectable oestradiol was found in the supernatant indicating that the contribution of the receptor-bound oestradiol to the total supernatant oestradiol concentration was unmeasurably low under the conditions used [i.e. $\leq 10\%$ of the total oestradiol (corresponding to approx. 5 pg of oestradiol in the assay tubes, which is the limit of the sensitivity of the method)].

The nuclear oestrogen receptor concentration decreased during the period between 0.5 h and 7.5 h after the oestradiol injection with the apparent half-life of approx. 140 min for the soluble and approx. 200 min for the insoluble fraction (Fig. 3b). The cytosol oestradiol concentration decreased at a similar rate whereas the cytosol receptor concentration rose to approx. 60% of its initial value by 7.5 h.

Cytosol receptor replenishment after oestradiol ($1\mu\text{g}$) injection

To distinguish whether the re-increase in the cytosol oestrogen-binding sites concentration within the first few hours after oestradiol administration is due to a process such as, e.g., activation or unmasking of the binding sites or to a synthesis of new 'receptor' molecules, rats were injected with oestradiol ($1\mu\text{g}$) and 0.5 or 6 h after with cycloheximide at an amount known to block the protein synthesis ($200\mu\text{g}/\text{rat}$; cf. Sarff & Gorski, 1971). At 1 h before killing, the

Fig. 3. Concentration of cytoplasmic oestradiol and the receptor distribution in rat uterus after injection of $1 \mu\text{g}$ of oestradiol (a) and the rate of decrease of the concentration of oestrogen-binding sites in uterine nuclear fraction *in vivo* (b)

(a) Concentrations of uterine cytoplasmic oestradiol and of cytoplasmic and nuclear oestrogen receptor were determined as described in the Materials and Methods section. The results shown are expressed as % of the concentrations of cytoplasmic oestrogen-binding sites in non-oestrogen-treated animals (mean $3.5 \text{ fmol}/\mu\text{g}$ of DNA); they represent means of three or four determinations (three uteri/determination) and are corrected for variations in DNA content. $\circ$, Cytoplasmic oestradiol; $\square$, cytoplasmic oestrogen-binding sites; $\triangle$, soluble nuclear oestrogen-binding sites; $\blacktriangle$, insoluble nuclear oestrogen-binding sites. (b) Results from (a) were plotted on a semilogarithmic scale: $\triangle$, soluble nuclear oestrogen-binding sites; $\blacktriangle$, insoluble nuclear oestrogen-binding sites. The half-lives estimated are 140 min for the soluble and 200 min for the insoluble nuclear oestrogen-binding sites.

cycloheximide-injected as well as control animals, received $1 \mu\text{Ci}$ of $L\text{-}[^{14}\text{C}]\text{leucine}$ intraperitoneally. The results (Fig. 4) showed that although protein synthesis was inhibited to at least 80% (degree of inhibition during 1 h before killing the animals) by the cycloheximide, there was no significant difference between cycloheximide-injected ($43 \pm 10\%$ s.d. of the initial content of cytosol oestrogen-binding sites) and control (i.e. only oestradiol-injected) animals ($48 \pm 11\%$ s.d. of the initial content of cytosol oestrogen-binding sites) in terms of cytosol oestrogen-binding sites recovery 6 h after the oestradiol administration. However, a large difference was observed in the concentration of cytosol oestrogen-binding sites between the cycloheximide-injected and control (only oestradiol-injected) rats when the drug was given 6 h after the hormone and the animals were killed 5 h later (11 h after oestradiol treatment). Cycloheximide when injected 0.5 h after oestradiol treatment was only slightly more efficient in inhibiting the oestrogen-binding-site replenishment by 11 h than when injected at 6 h. The same results were obtained when the hormone was injected intraperitoneally instead of subcutaneously. The results were confirmed in repeated experiments (results not shown).

Cytosol-receptor variations after injection of $0.1 \mu\text{g}$ of oestradiol

Since the above results did not agree with the pattern of the supernatant receptor replenishment

described by Sarff & Gorski (1971), the relevant part of their experiments was repeated by injecting $0.1 \mu\text{g}$ of oestradiol intraperitoneally into 22–23-day-old rats.

Again (Fig. 5), cycloheximide given 6 h after oestradiol, inhibited the oestrogen-receptor replenishment between 6 and 12 h after the hormone injection. In agreement with Sarff & Gorski (1971) a lower concentration of oestrogen-binding sites was detected in animals already given the drug at 0.5 h after the hormone, compared with those only given cycloheximide 6 h after oestradiol administration; however, this difference was much smaller than that reported by these authors. Again, repeated experiments (results not shown) gave similar results.

Discussion

Administration of a high dose ($1 \mu\text{g}/\text{rat}$) of oestradiol led to a rapid disappearance of a large proportion of the oestrogen receptor from the uterine cytoplasmic fraction. Only about one-half of the receptor lost from the cytoplasm could be recovered in the nuclear pellet by the exchange method (Anderson *et al.*, 1972).

The missing part of the receptor is either due to underestimation of the nuclear oestrogen binding (if e.g. a part of the nuclear binding sites formed under the conditions *in vivo* was not exchangeable during the 0.5 h at 37°C), or was destroyed (inactivated) within the 0.5 h after oestradiol administration. It is unlikely that a significant quantity of occupied receptor sites was present in the cytosol fraction since

Fig. 4. Effect of cycloheximide on the replenishment of rat uterine supernatant oestrogen-binding sites after injection of 1 µg of oestradiol

The rats (three to four/group) were injected subcutaneously with 1 µg of oestradiol in 0.5 ml of saline and killed at the times indicated. Two groups of three rats received in addition cycloheximide (200 µg in 0.25 ml of saline) subcutaneously either 0.5 or 6 h after oestradiol. Each uterus was homogenized in 0.6 ml of homogenizing medium and the cytoplasmic oestrogen receptor concentrations in individual uteri were determined by Scatchard (1949) plot analysis as described in the Materials and Methods section (method II). The results are expressed as % of uterine cytoplasmic oestrogen-binding site concentration in non-oestradiol-treated rats and are corrected for variations in DNA contents. The results are represented as means of three or four uteri $\pm$ s.d. (vertical bars); the number of uteri is indicated in the diagram. ○, Oestradiol alone at 0 h; ●, oestradiol at 0 h, cycloheximide at 0.5 h; ◐, oestradiol at 0 h, cycloheximide at 6 h.

no detectable receptor-bound oestradiol was found by the competitive binding technique. A similar loss of a part of the cytosol oestrogen receptor was reported by Milgrom *et al.* (1973) and Feherty (1972).

Experiments involving the use of a protein-synthesis inhibitor (cycloheximide) led us to reconsider the sequence proposed by Sarff & Gorski (1971) for cytoplasmic receptor replenishment after oestradiol injection, according to which the entire synthesis of the new 'receptor' molecules occurs within the first 6 h and the subsequent increase in oestrogen-binding capacity is due to 'activation' independent of continuing protein synthesis. In the present experiments, at 6 h after administration of 1 µg of oestradiol/rat, approx. 50% of the initial content of free cytosol oestrogen-binding sites were recovered in the cytoplasm by a process that may involve their reactivation and/or unmasking and that

Fig. 5. Effect of cycloheximide on the replenishment of rat uterine supernatant receptors after injection of 0.1 µg of oestradiol

The rats (two to three/group) were injected intraperitoneally with 0.1 µg of oestradiol in 0.5 ml of saline and killed at the times indicated. Each uterus was homogenized in 0.6 ml of the homogenizing medium, and the cytoplasmic receptor concentrations in individual uteri were determined by the Scatchard (1949) plot analysis as described in the Materials and Methods section (method II). Two groups of three rats received in addition cycloheximide (200 µg in 0.25 ml of saline) subcutaneously either 0.5 or 6 h after oestradiol treatment. The results are expressed as % of the uterine cytoplasmic oestrogen-binding site concentration in non-oestradiol-treated rats and are corrected for variations in DNA contents. Each point represents the receptor concentration found in a single uterus; points corresponding to the same experimental group are joined. ○, Oestradiol alone at 0 h; ●, oestradiol at 0 h, cycloheximide at 0.5 h; ◐, oestradiol at 0 h, cycloheximide at 6 h.

is not blocked by cycloheximide. The second stage of replenishment from 6 h onwards was at least partly dependent on continuing protein synthesis. When the injected dose of oestradiol was only 0.1 µg, a substantial part of the cytoplasmic receptor increase observed between 4 and 12 h after was still inhibited by cycloheximide given 6 h after oestradiol treatment, and was therefore presumably due to protein synthesis, although a smaller contribution of the 'activation' of pre-formed 'receptor' molecules cannot be excluded.

Quantitative comparison of the maximum concentration of nuclear oestrogen-binding sites reported here with those found under similar circumstances by Anderson *et al.* (1972) shows that their values are approximately three times higher. In view of the fact that the above authors suggest an increase

in nuclear-binding capacity after oestradiol injection (maximum value approx. 1.5 pmol/uterus) that exceeds by approx. 65% the total cytosol receptor-binding capacity (approx. 0.9 pmol/uterus) of the non-injected rat uterus (see, e.g. Gorski *et al.*, 1970), the lower estimates appear more likely to be valid. In agreement with Anderson *et al.* (1972), the presence of oestrogen receptors in the nuclear fraction of immature non-oestrogen-treated rat uterus has been observed. This result may be due to the presence of endogenous oestrogens (Meijs-Roelofs *et al.*, 1973), or to a contamination by a small portion (approx. 10%) of the cytoplasmic receptor, e.g. during homogenization.

An interesting phenomenon observed in the experiments *in vivo* was the apparent coexistence of free oestrogen-binding sites and unbound oestradiol in the supernatant fraction whereas a receptor-oestradiol complex could not be detected; however, the free oestradiol pool may be partly or entirely extracellular.

The nuclear oestrogen receptors studied in this work were of two categories: the KCl-extractable form and the residual form. Most work up to now has been concerned with the KCl-extractable nuclear oestrogen receptors, although a certain amount of the high-affinity bound oestrogen has always remained in the residual pellet depending on the conditions of extraction. The presence of a distinct non-KCl-extractable fraction of bound oestrogen in rat uterine nuclei has been reported by De Hertogh *et al.* (1973); a similar 'non-soluble' residual oestrogen receptor from chick liver nuclei was studied in detail by Lebeau *et al.* (1973, 1974). The experimental evidence suggests that *in vivo* there may exist an equilibrium between the two forms of the receptor that reflects the (always arbitrary) extraction conditions. The possibility of interconversion of the

two forms of the receptor is suggested by the variations of their relative concentrations under conditions both *in vitro* and *in vivo*.

References

- Anderson, J., Clark, J. H. & Peck, E. J., Jr. (1972) *Biochem. J.* **126**, 561–567
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- De Hertogh, R., Ekka, E., Vanderheyden, J. & Hoet, J. J. (1973) *J. Steroid Biochem.* **4**, 313–320
- De Sombre, R. E. (1970) *Advan. Biosci.* **7**, 16
- Feherty, P. (1972) Ph.D. Thesis, University of London
- Feherty, P., Robertson, D. M., Waynforth, B. & Kellie, A. E. (1970) *Biochem. J.* **120**, 836–845
- Gorski, J., Sarff, M. & Clark, J. (1970) *Advan. Biosci.* **7**, 5–15
- Jensen, E. V., Suzuki, T., Numata, M., Smith, S. & De Sombre, E. R. (1969) *Steroids* **13**, 417–427
- Lebeau, M. C., Massol, N. & Baulieu, E. E. (1973) *Eur. J. Biochem.* **37**, 294–300
- Lebeau, M. C., Massol, N. & Baulieu, E. E. (1974) *FEBS. Lett.* **43**, 107–111
- Mešter, J. & Baulieu, E. E. (1972) *Biochim. Biophys. Acta* **26**, 236–244
- Mešta, J. & Robertson, D. M. (1971) *Biochim. Biophys. Acta* **230**, 543–548
- Mešter, J., Robertson, D. M., Feherty, P. & Kellie, A. E. (1970) *Biochem. J.* **120**, 831–836
- Mešter, J., Robertson, D. M. & Kellie, A. E. (1971) *J. Steroid Biochem.* **1**, 1–10
- Meijs-Roelofs, H. M. A., Uilenbroek, J. T. J., De Jong, F. H. & Welschen, R. (1973) *J. Endocrinol.* **59**, 295–304
- Milgrom, E., Luu Thi, M. & Baulieu, E. E. (1973) in *Protein Synthesis in Reproductive Tissue* (Diczfalusy, E., ed.), pp. 380–397, Bogtrykkeriet Forum, Copenhagen
- Sarff, M. & Gorski, J. (1971) *Biochemistry* **10**, 2557–2563
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672